HPLC DATA SHEET

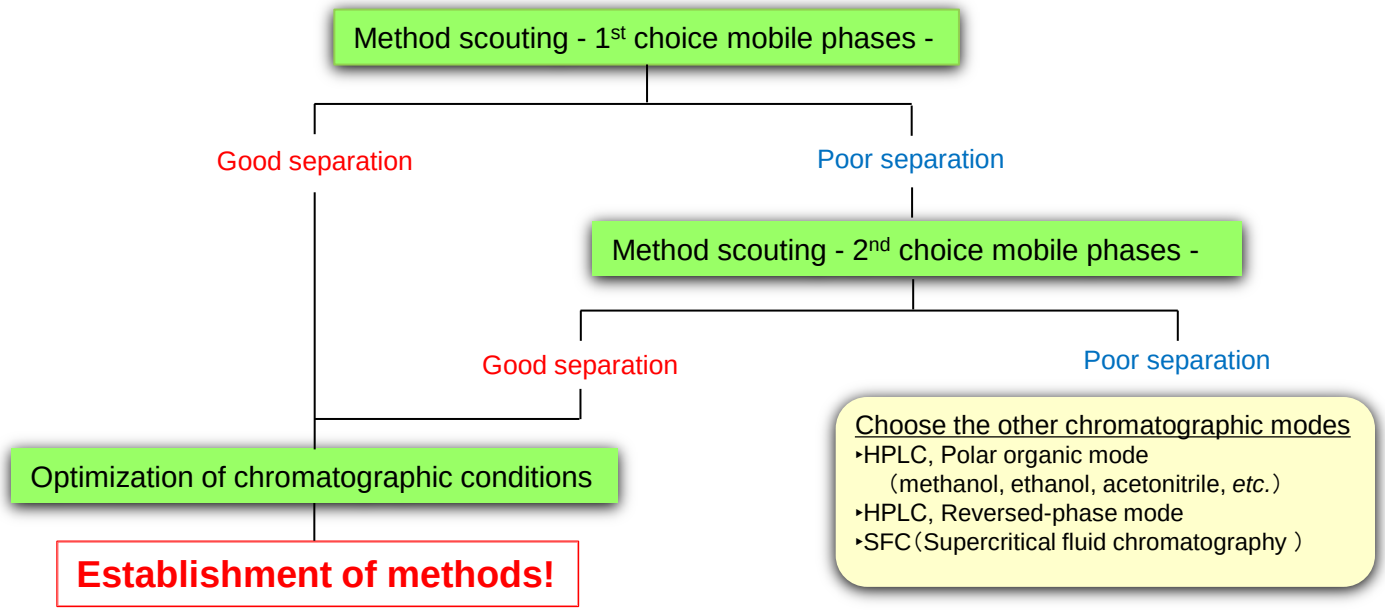
Efficient method development for chiral separation by using CHIRAL ART columns

U190520AE

Liquid chromatography using polysaccharide-based chiral separation columns is a popular method for the separation of enantiomers. However, it has been very hard to find the correct chiral separation column and mobile phase condition for achieving an acceptable chiral separation.

CHIRAL ART columns (including four immobilized types and two coated types) exhibit excellent chiral separation performance for a wide range of racemic compounds. In this report, we introduce the efficient method development for the separation of enantiomers via a high throughput method scouting using the CHIRAL ART columns.

Workflow of method development for chiral separation



Method scouting strategy for chiral separation

<Column>

CHIRAL ART

Immobilized type

① Amylose-SA
② Cellulose-SB
③ Cellulose-SC
④ Cellulose-SJ

Coated type

⑤ Amylose-C Neo
⑥ Cellulose-C

×

<Mobile phase>

1st choice

❶ A) *n*-hexane
B) 2-propanol

❷ A) *n*-hexane
B) ethanol

2nd choice

❸ A) *n*-hexane
B) THF*

❹ A) *n*-hexane
B) MTBE*
(methyl *tert*-butyl ether)

❺ A) *n*-hexane
B) ethyl acetate*

* Applicable only for immobilized type columns

Gradient elution

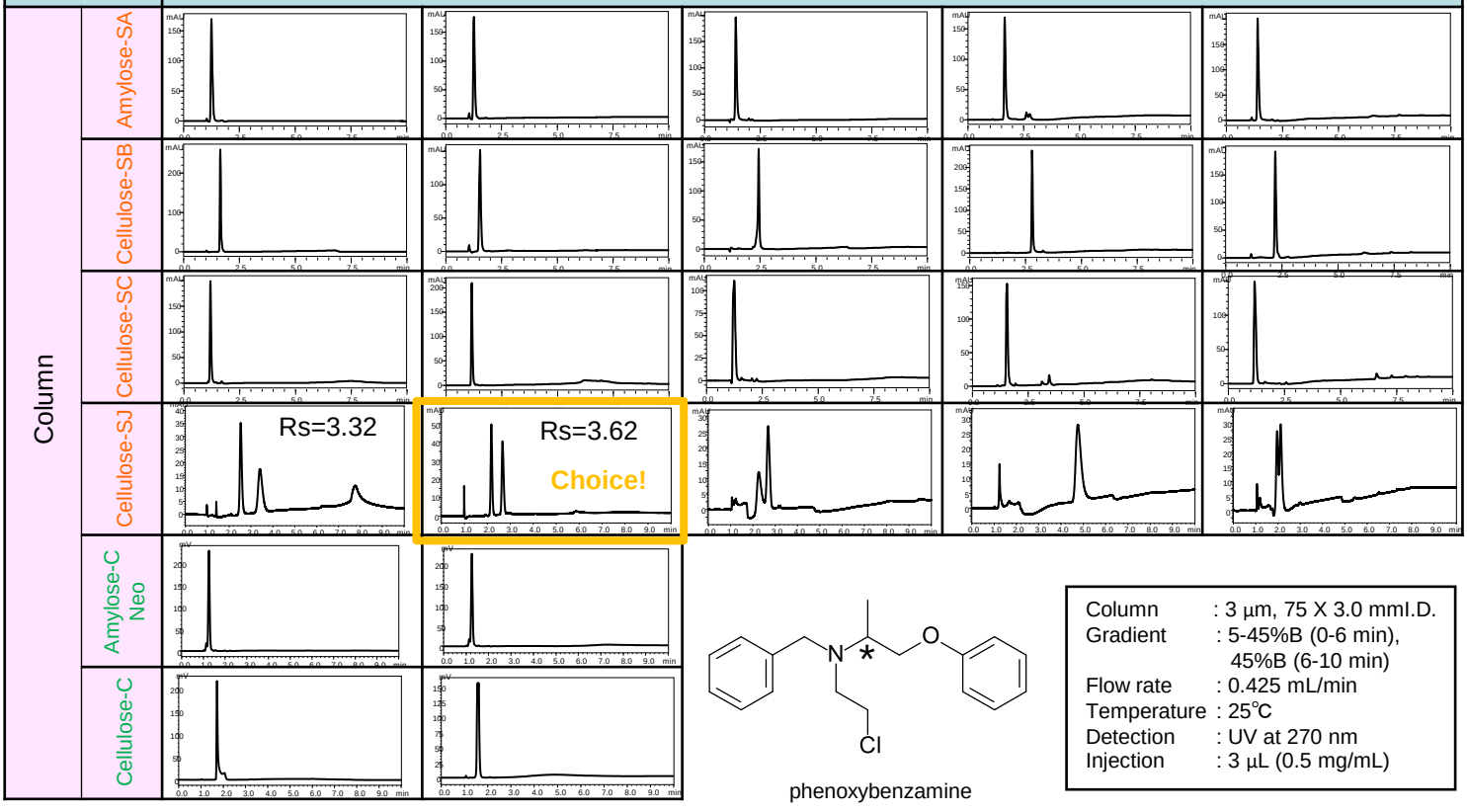
Additive

Basic additives	:	Diethylamine (DEA), *etc.*	Usually 0.1%
Acidic additives	:	Trifluoroacetic acid (TFA), Formic acid, *etc.*	

Example of method scouting by using CHIRAL ART columns

The method scouting for the chiral separation of phenoxybenzamine was carried out. As shown in below chromatograms, the baseline separation of phenoxybenzamine was achieved using Cellulose-SJ column. For the further method development, the combination of Cellulose-SJ and *n*-hexane/ethanol containing 0.1% DEA was chosen according to resolution and peak shape.

Eluent A	*n*-hexane				
Eluent B	2-propanol	ethanol	THF	MTBE	ethyl acetate
Additive	0.1% DEA				



phenoxybenzamine

Column : 3 µm, 75 X 3.0 mmI.D.
Gradient : 5-45%B (0-6 min), 45%B (6-10 min)
Flow rate : 0.425 mL/min
Temperature : 25℃
Detection : UV at 270 nm
Injection : 3 µL (0.5 mg/mL)

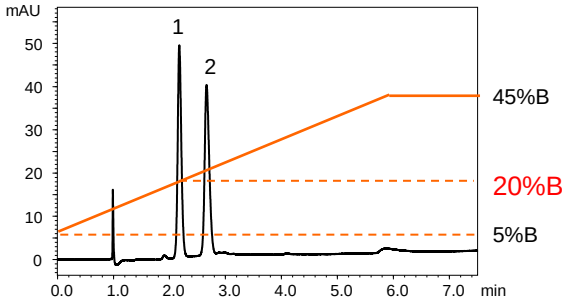
Optimization of chromatographic conditions

On the basis of the scouting results, the chromatographic conditions were optimized depended on the purpose of the chromatography experiment. To operate in an isocratic elution mode, it is appropriate to decrease the proportion of the polar organic solvent component in mobile phases by 10-15%pt from the proportion at the first eluting peak under gradient conditions. For a rapid analysis, short columns with 3 µm particles are recommended. For a preparative purpose, the method optimization and loading studies by using 250 X 4.6 mmI.D. columns with 5 µm particles make it easy to scale up to preparative columns.

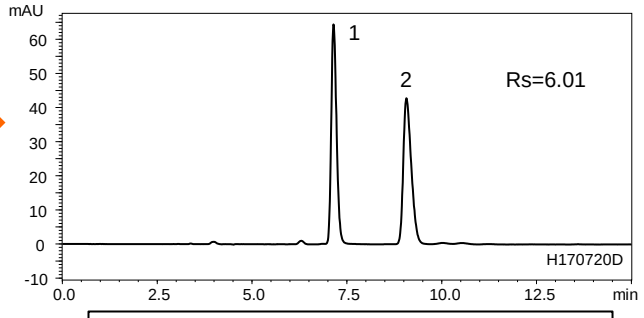
Optimized conditions
- Eluent (gradient⇒isocratic)
- Particle size, Column size
- Flow rate
- Injection volume, *etc.*

Chosen chromatogram via the method scouting

Column : CHIRAL ART Cellulose-SJ 3 µm, 75 X 3.0 mmI.D.
Eluent : A) *n*-hexane/DEA (100/0.1)
B) ethanol/DEA (100/0.1)
5-45%B (0-6 min)



Optimization



Column : CHIRAL ART Cellulose-SJ 5 µm, 250 X 4.6 mmI.D.
Eluent : *n*-hexane/ethanol/DEA (95/5/0.1)
Flow rate : 1.0 mL/min
Temperature : 25 ℃
Detection : UV at 270 nm
Injection : 5 µL (1.0 mg/mL)